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(54) Title: SESSOR AND SENSING METHOD FOR DETECTION AND PROCESS CONTROL

(57) Abstract: A sensor for electrochemically or optically determining a desired species, the sensor comprising at least one electrode or optical path, wherein the electrode or optical path is modified for specific accumulation of the desired species, by means of a modifying agent which is adapted to selectively and substantially reversibly bind the species to be detected and to release the species at a characteristic voltage or give an absorbance measurement at a characteristic optical wavelength, and wherein the modifying agent is associated with at least part of the surface of the electrode or optical path by physical or chemical anchoring. A method of fabricating and using such a sensor, particularly in harsh media such as whole blood and other body fluids, brackish water, industrial environments and the like.

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**SENSOR AND SENSING METHOD**  
**FOR DETECTION AND PROCESS CONTROL**

5 The present invention relates to a novel sensor for detecting a desired chemical species, novel components thereof, a sensing method for detection of a desired species, and the use thereof in medical, environmental and industrial applications and to a method for the control of industrial processes for the reaction of a desired chemical species. More particularly the invention relates to a sensor for  
10 use in harsh media such as whole blood and other body fluids, brackish water, industrial environments and the like, for determination and speciation of species such as heavy metals, pesticide residues and the like comprising a sensor including a modifying agent for mobilisation and transport of a desired species, a method for determination of a desired species, as a contaminant or otherwise, a  
15 method for the control of industrial processes for the reaction thereof, and the use of the sensor and methods in medical applications and in environmental and industrial applications where heavy metals, pesticide residues and the like are employed and discharged into the environment.

20 Chemically harsh media such as whole blood, brackish water, and fluids used in industrial processes present a particularly difficult environment for accurate sensing. There are nevertheless many circumstances where an accurate indication of trace species such as contaminants might be desirable. Similarly, contaminated land clean up poses an ever increasing challenge in which  
25 understanding the nature of contamination on previously developed land is critical.

Test methods exist for determining trace species and contaminants, but in the harsh media involved these typically involving taking samples and submission to  
30 sophisticated analytical techniques, as off site processes, which may require

treatment of the sample, for example to separate a target species from the harsh sample medium before a delicate test is performed. This can often lead to turn arounds of one to two weeks with significant costs. Moreover numerous different tests must be conducted to determine any of a number of contaminants  
5 which may be present.

There is therefore a need for an improved sensor and test method which yields immediate results from a sample at the point of collection or in situ, thereby leading to a major reduction in time, analysis expense and complication. Such  
10 device is considered below by way of illustration in the context of detection of industrial waste, in particular heavy metals such as chromium, and oil residues, liquid effluents such as pesticides and the like. However it will be understood that the particular problems posed by harsh media such as whole blood, brackish water, industrial environments and the like are relevant, and the advantages  
15 offered by the invention are generally applicable in all such environments.

A further application is envisaged in controlling industrial processes such as metal finishing, metal oxides, wood preservatives, and the leather tanning industry which uses over 750,000 tonnes of chromium chemicals per year, and  
20 the like, to optimise processes, minimising consumption of heavy metals, and eliminating at least in part the metal wastage to effluent and disposal issues.

Although there are a wide variety of expensive, time-consuming laboratory-based techniques available for the determination of chromium and its speciation  
25 in water including AES, AAS, IPM-MS, HPLC, CE-coupled IPM-MS etc., these are not particularly suitable for many industrial processes. Electroanalytical methods described in literature for determining Cr (111) include limited references to ion-selective electrodes characterised in aqueous solutions. Chromium speciation in industrial effluents has been studied using time-  
30 consuming and inadequate laboratory based techniques such as 1,5 diphenyl-

carbazide based spectrophotometry, extraction with methyl isobutyl ketone and co-precipitation with iron and bismuth salts. In presence of organic complexes, all three methods gave erroneous results requiring empirical corrections. The current state of art, although extensive, is complex and inadequate for the 5 development of a commercial instrument.

One potentially interesting electro-chemical sensing technique comprises programmed absorptive stripping voltammetry. This method involves accumulating species on one or more electrodes of a sensor which is then 10 provided with electrical contact and scanned through a voltage or current range.

Amperometric detection shows peak current flow, and calibration of the sensor indicates characteristic voltages at which given species are desorbed from the electrode. When the voltage applied across the electrode is held constant, measurement of the current yields a result that can be converted electronically to 15 the concentration of the detected species. The existing technologies generally involve gold or mercury electrodes and have the significant disadvantages in that they are relatively expensive, require the use of strong acids and are easily blocked in highly mixed organic and inorganic process liquors.

20 US 6,090,269 (Yissum RDC) discloses a stripping voltammetry method using gold electrodes, having SAM's thereon, as a result of thiol binding to the electrode, however these are unstable in harsh media.

25 WO 96/24840 (Ecossensors Limited) discloses an enhanced sensitivity or specificity anodic stripping voltammetry method comprising a working electrode and a chemical species incorporated in a layer over or close to the surface of the electrode or incorporated into the electrode. The chemical species enables detection of metals in the presence of interfering species, e.g lead in presence of copper. Other techniques use ligands in the sample solution which are specific to

a species to be detected and freely accumulate these species, providing some improvement in sensitivity or specificity.

Potentiometric ion-selective electrodes (ISEs) non-destructively measure ionic activities rather than concentrations. The measurements are made in equilibrium conditions. In several situations, measurements of activities are more meaningful than measurements of total concentrations as these give an indication of the active form of the compound. A classic example is the significance of calcium ionic activity rather than total concentration in blood electrolyte measurements.

The current limitation is the sensitivity of the method, which, realistically, only goes down to micromolar levels using established techniques. However research is underway to lower limits of detection and the present invention will contribute towards this goal.

Potentiometric polymer membrane ISEs for over 60 analytes have been reported over the past decade<sup>i</sup>. Membranes of ionophore based ISEs traditionally consist of a polymeric phase (typically poly(vinylchloride)) as polymer matrix, lipophilic ion-exchanger sites, and a plasticiser which also acts as a solvent for a lipophilic ionophore. The membrane is placed between two aqueous phases, the sample and the inner filling solution (usually an approximately millimolar concentration of the primary analyte ion), which is in contact with an inner reference electrode, usually Ag/AgCl in traditional ion-selective electrodes. With trends towards miniaturisation, the ion-selective membranes are deposited on the conductor, replacing the conventional inner filling solution, giving coated wire electrodes.

Elimination of the internal filling solution is the first step towards miniaturisation. The ultimate stage in miniaturisation is deposition of the electroactive material directly on the insulator of a field-effect transistor giving an ion-selective field effect transistor (ISFET). Traditionally, limits of detection at micromolar levels have limited the scope of ion-selective potentiometry to analysis requiring higher levels of detection. Recently, there has been a renewed

surge of interest in ISEs as sub-pico molar levels of detection have been achieved by optimisation of the electrodes<sup>ii,iii</sup>. The reason for the moderate detection limit of ion-selective electrodes, as compared to equivalent optodes, has been rationalised to be due to the leaching of the primary ions from the inner filling 5 solution into the sample which builds up on the aqueous surface layer of the membrane, the bulk of which contains a much lower concentration of the ion. Bakker, Sokalsky et al.<sup>i,ii</sup> induced lower limits of detection and obtained 'true' selectivity coefficients by building a concentration gradient in the membrane to drive the released primary ion backwards through the membrane by using 10 buffered inner filling solutions with low primary ion but high interfering ion concentrations. Lindner et al.<sup>iv</sup> used galvanostatic control and flow-through conditions to improve the detection limits.

Accordingly there is a need for a sensitive and specific means for sensing target 15 species in harsh media, for example target chemicals in body fluids, heavy metals such chromium in environmentally contaminated or industrial etc. There is a particular need for a method for contaminant detection which is suitable for use in an on-line process control method. There is moreover a need for an improved method for sensing of heavy metals, oil and pesticide residues, and the like.

20

We have now surprisingly found that stripping voltammetry methods, and indeed optical equivalents thereof, can be further enhanced in terms of specificity and sensitivity by modifying the electrode or optical contact with a chemical agent which is anchored securely at the electrode and which selectively and reversibly 25 bonds to the species to be detected thereby controlling the mobilisation and transport thereof.

Accordingly in the broadest aspect of the invention there is provided a sensor for electrochemically or optically determining a desired species, the sensor 30 comprising at least one electrode or optical path, wherein the electrode or optical

path is modified for specific accumulation of the desired species, by means of a modifying agent which is adapted to selectively and substantially reversibly bind the species to be detected and to release the species at a characteristic voltage or give an absorbance measurement at a characteristic optical wavelength, wherein  
5 the modifying agent is associated with at least part of the surface of the electrode or optical path by physical or chemical anchoring.

The modifying agent is chosen so that it has specific binding sites for the desired species. When a potential or optical source is applied the species are loosely  
10 bound to the agents surface. Sweeping the potential or wavelength leads to desorption of the ionic species present. The sensor of the invention has the additional benefit that it can distinguish between oxidation states, for example Cr (III) and the highly toxic Cr (VI), as these ions are selectively desorbed at different voltages or wavelengths.

15

The invention is particularly adapted to use in harsh media such as whole blood and other body fluids, brackish water, industrial fluids or contaminated environments and the like, or in testing samples taken directly from such harsh media without a need for remote testing or sample pre-treatment. The modifying  
20 agent is adapted to bind the species to be detected and to release the species at desired moment for analysis. Thus the sensor inherently performs both a separation function, in which the target species is effectively separated from the harsh environment of the sample by the action of the modifying agent, and a sensor function.

25

The sensor may be electrochemical or optical. An electrochemical sensor may be amperometric or potentiometric. The role of the modifying agent in amperometric sensors is to ensure a high concentration of the analyte (species to be detected) in the vicinity of the electrode, detection occurs via a redox reaction  
30 of the analyte at the electrode surface; consequently, equilibrium conditions are

not necessary and the electrode need not comprise an inner sensing chamber or region.

In the amperometric, non-equilibrium mode, sub-picomolar levels of detection  
5 are achievable, using adsorptive stripping techniques, for species capable of undergoing redox reactions. The species to be detected is pre-concentrated on a modifying agent of moderate selectivity by applying a potential opposite to the charge on the analyte. Reversing the potential then strips the species. Very high selectivities can be obtained by combining modifying agent-based pre-  
10 concentrations and stripping. The stripping potentials are characteristic for each species, thereby enabling species discriminations as well. In order to enhance selectivity, modifying agents with appropriate binding constants are deposited either physically (screen-printed) or chemically attached to the electrode surface.

15 In a particular advantage the sensor of the invention serves to integrate the sensing and separation of desired species by incorporation of separation on the sensor surface achieved by differing electrophoretic mobilities of the species. Moreover the ability of the sensor to speciate in situ revolutionises the uses thereof and provides an excellent sensor for use in process control.

20

The sensor may comprise exclusion means for excluding non desired species from the surface of the electrode or optical source, such means selected from size-specific exclusion means about the electrode or optical source and remote therefrom, to prevent access of large particles such as dust, soil, cells etc, or  
25 exclusion agents about the electrode or optical source and remote therefrom, for example pepsin for prevention of protein fouling and the like.

The sensor may comprise an additional layer of material such as porous polymer with tuneable porosity, preferably gradient porosity, mechanical stability and  
30 resistance to fouling. PolyHIPE is particularly suitable for improving the

analytical capabilities of electrochemical separation and detection. Physically, the material is inert, easy to manipulate, has tuneable porosity, can be cast as open or closed cell structures, and is mechanically stable.

5 The modifying agent and any exclusion means or agents may be chemically or physically anchored to the electrode or optical source or remote therefrom and thereabout.

Chemical anchors may include any chemical modifying groups which are known  
10 for anchoring chemical substrates to metal, composite or polymer or like surfaces. Preferred anchoring groups include lipophilic groups such as C<sub>4-20</sub> aliphatic or alicyclic saturated or unsaturated hydrocarbon groups for example C<sub>8</sub> octyl groups and the like.

15 Physical anchors may comprise any known means for physically anchoring a chemical substrate to metal, composite, polymer or like surfaces. Preferred physical anchoring means comprises a porous open or closed cell membrane or film applied at or about the surface and having electrode or optical source modifying agent anchored in turn chemically or physically within its pores.  
20 Physical anchoring of modifying agent is achieved with use of pore interconnects of diameter which preclude release of modifying agent but allow access of the species to be detected. Chemical anchoring of modifying agent is achieved with use of groups or substituents having an affinity for the porous material. Preferred porous open cell structures include high internal phase  
25 emulsion polymers (Poly-HIPE or PHP), solgels, electrically conducting or optically transparent zeolites and the like. Preferably anchoring means comprises PHP membrane of thickness 1 micron – 5 mm, preferably 0.01 – 3 mm and having pore size in the range up to 100 micron and interconnect size in the range up to 10 micron. Suitably modified PolyHIPE therefore provides an excellent

material for developing integrated amperometric electrochemical systems combining separation, detection and compatibility.

It is a particular advantage that the mechanical anchoring means may be adapted  
5 for use with different modifying agents specific for detection of a range of different species, thereby simplifying the process for sensor manufacture.

A modifying agent according to the invention may be any known or novel agent which is capable of selectively and reversibly binding a desired species to be  
10 detected. Preferably modifying agents are selected from ionophores, ligands, enzymes and other multi-dentate species providing reversible binding by affinity such as charge delocalisation, for example hydrogen bonding, van der Waals forces and the like.

15 More preferably modifying agents are selected from compounds of formula  
 $(R)_n - A$

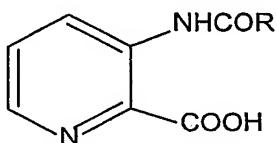
wherein n is 0 – 2 and R is a lipophilic or other group suitable for stably  
20 binding with high affinity to the electrode surface or a surface layer such as polyHIPE or PVC surface membrane; when n is 0, the ligand is physically anchored, for example caged in membrane pores

and wherein A is a C<sub>1-36</sub> aliphatic or alicyclic saturated or unsaturated or  
25 aromatic hydrocarbon moiety optionally including one or more heteroatoms selected from N and S and the like, and optionally substituted by functional groups including halo, hydroxy, cyano, amino, carbonyl, and the like and optionally including one or more ether, amino or thioether linkages and having m binding sites for  
30 accumulating a desired cationic species, wherein m is 1 – 6, preferably 1 – 4.

The sensor may be used for detection of any desired species, for example selected from the heavy metals, pesticides, oil residues and the like. In a preferred embodiment the sensor is adapted for the detection of chromium species and in particular for the detection of Cr(III) speciated with respect to Cr(VI).

Preferred modifying agents for detection of chromium include ligands comprising modified 2, 3 pyridine dicarboxylic acid of general formula I

10



15

Fig 1

which may optionally be substituted at the 4, 5 and 6 positions by C<sub>1-8</sub> saturated or unsaturated hydrocarbon moieties, halo and the like or fused polyaromatic systems.

20

Preferred modifying agents for detection of lead include ligands comprising modified N, N' substituted aliphatic diamides such as N,N'-dioctadecyl-N,N'-dipropyl-3,6-dioxaoctandiamide (Fluka).

25

Preferred modifying agents for detection of potassium K<sup>+</sup> include ligands selected from the class of the mycin antibiotics such as valinomycin.

30

Modifying agents may also be employed comprising ionophores for detection of hydrophilic species such as Calcium Ca<sup>2+</sup>, sodium Na<sup>+</sup>, magnesium Mg<sup>2+</sup> and the like, or for detection of hydrophobic species such as ionophores for chiral and

achiral aryl and alkyl ammonium cations, or for detection of other environmental pollutant cations such as ionophores for Cd<sup>2+</sup>, Hg<sup>2+</sup> and the like.

Alternatively the modifying agent is selected from known enzymes, ionophores,  
5 sequestering agents, oligomeric species and the like.

Preferably the modifying agent comprises moderate species-agent binding affinity (of the order of -log K = 4-7) to ensure pre-concentration and allow stripping within the voltage or wavelength window of the electrode or optical  
10 system. Suitably agents also provide moderate selectivity, either by means of specificity or by means of stripping potential or wavelength of each species being adequately separated or having "finger print" patterns.

In addition the agent provides sufficient affinity, or lipophilicity to minimise  
15 leaching of the agent from the electrode or optical source surface.

The sensor of the invention may comprise any known or novel electrode or optical source and detection arrangement. Preferably an electrochemical sensor comprises one or more electrodes. Electrodes may be screen printed on to a  
20 suitable carrier such as a plastic carrier using conductive inks as known in the art or may comprise a composite electrode as known in the art. Electrodes are typically comprised of carbon, such as graphite.

The sensor may be multi use or may be for disposable single use. The sensor  
25 may be suited for immersing in a sample cell, or may be in the form of a probe for inserting directly into material to be determined.

Alternatively the sensor may be mounted as an array for example in a liquid effluent stream to provide continuous measurement of heavy metal  
30 concentrations or in a reaction vessel to provide continuous measurement of

## 12

heavy metal concentrations in the course of their reaction. This sensor may advantageously be based on end of capillary, electrochemical detection combined with capillary electrophoresis. In a particular advantage this combination is well suited to on-line and field based instrumentation, whereby the instrument may be  
5 suitable for portable electroanalytical systems. A detector cell may be of any suitable geometry and is typically of wall-jet configuration which reduces contamination of the electrode surface by minimising the impact time of the analyte solution plug. This allows simultaneous detection of pH. The on-line instrument suitably provides excellent life time, response time, sensitivity,  
10 hysteresis and interferences in both industrial and environmental samples.

Optical sensors may similarly comprise any single or multi use sensor which may be disposable or otherwise. An optical sensor may comprise a cell having optical source and detection means or may comprise an optical fibre for conducting light  
15 of desired wavelength into a sample to be detected. Preferably optical sensing is with UV light.

In a further aspect of the invention there is provided a method for the manufacture of a sensor as hereinbefore defined comprising providing at least  
20 one electrode or optical path, applying a layer of modifying agent thereabout, and anchoring by chemical or physical means, applying exclusion means thereabout and anchoring, and providing electrical contacts or optical source. The sensor may be provided as an array or as a single contact sensor.

25 In a further aspect of the invention there is provided a novel electrode, modified with use of a modifying agent as hereinbefore defined, preferably with use of a Cr (III) selective agent, for Cr (III) detection or with use of a Cr (VI) selective agent, for Cr (VI) detection. Cr (VI) is highly toxic and therefore of environmental concern.

In a further aspect of the invention there is provided a method for species detection with use of a sensor as hereinbefore defined comprising providing or obtaining a sample to be detected in fluid phase, applying an electrical current or optical source and scanning through a pre-determined range to a stripping potential or wavelength, maintaining the current or optical *source* at that potential or *wavelength* and detecting or measuring the current evolved or absorbance, optionally with conversion thereof to indicate concentration of species.

5

10 The method is suitable for the measurement of species as hereinbefore defined down to 0.1 ppm of heavy metal or  $10^{-9}$  mg/litre of pesticides such as organophosphate. The method is suitable for detection of heavy metals such as chromium, arsenic, elemental cadmium, lead or zinc and other metals, for the detection of pesticides such as organophosphates, for the detection of oil residues

15 and the like.

Measurement may be of liquid samples directly or of analyte extracted from soil or from industrial process streams or effluent and made up as a sample. Extracts may be conveniently obtained by subjecting to ultrasound to break down signal

20 distorting contaminants, and to break down polymerised species to detectable form.

In the case that the species to be detected is volatile, detection is with use of a solid phase adsorptive column as sample cell.

25

In a further aspect of the invention there is provided a method for the regeneration or priming of a sensor as hereinbefore defined, comprising the (re)introduction of modifying agent. The method may be useful for changing the specificity of the sensor to detect a different species, or simply regenerating the

30 existing sensor for prolonged lifetime.

In a further aspect of the invention there is provided a method for process control of an industrial process for the introduction and reaction of a given species, comprising monitoring the species with use of the sensing method and sensor as hereinbefore defined, in situ in real time operation, periodically obtaining values for the concentration of species in a given location and deriving parameters to maintain or alter the process controls for continued efficient operation. The method involves the use of suitable software and instrumentation to receive and process signals and relate to a calibration logarithm to produce concentration values.

In a further aspect of the invention there is provided a method for effluent control or contaminant detection with use of the method and sensor as hereinbefore defined.

In a further aspect of the invention there is provided the use of a method or sensor as hereinbefore defined in electroplating or metal finishing, timber preservation, pigmentation, leather tanning, contaminant detection, nutrient development, heavy metal manufacture and the like.

The invention is now illustrated in non limiting manner with reference to the following examples and figures. The examples are explored in particular in relation to detection of heavy metals such as chhromium, but it will be appreciated that these are merely illustrative of the application of the principles of the present invention to detection of a wide range of target species in a wide range of harsh media, such as whole blood and other body fluids, brackish water, industrial processes industrially or otherwise contaminated environments and the like.

Example 1 – Identification of Modifying Agent

Ligands were selected in turn and made up in a solution with the desired species, Cr(III). Binding Constraints  $\beta_1$  and  $\beta_2$  for CrIII were determined by  
 5 potentiometric titrations and compared with literature values.

The results are shown in Table A.

Table A

10

	<b>Ligand</b>	$\beta_1$	$\beta_2$
1	2,6- Pyridine dicarboxylic acid	4.52	7.64
2	8-Hydroxy quinoline	9.76	18.24
3	Picolinic acid (2-pyridine carboxylic acid)	4.76	9.14
4	n-(2-carboxyphenyl)iminodiethanoic acid	9.40	
5	Pthalic acid	5.52	10.00
6	2,3-Pyridine dicarboxylic acid	4.71	7.08
7	Tartaric acid	3.97	6.84
8	p-toluenepridylsulfonamide	3.3	12.3

Solutions including the ligands were then subjected to anodic stripping  
 15 voltammetry, a result is shown in Fig 1. Signal transduction was obtained either electrochemically or optically to detect a characteristic species and calibration curves obtained.

## 16

Speciation plots with Ligand 8 at 10mM dm<sup>-3</sup>, 5mM dm<sup>-3</sup>, 0.1mM dm<sup>-3</sup>, shown in figure 2, reflect the higher basicity of the pyridyl nitrogen.

Exploratory stripping experiments with ligand 8 at pH 6 gave the results shown  
5 in Figure 3. (note that ligand is not L<sup>-</sup> at this pH)

Example 2 – Preparation of a Sensor

Suitable ligands were immobilised on a robust polymer of controllable porosity  
10 (Poly HIPE) and attached to ceramic screen-printed electrodes to enable usage even in harsh media or as a monolith in a dip-type sensor. An SEM of the Poly HIPE material is shown in figure 4, and a schematic of the sensor in Figure 5.

Sensors were constructed of the following generic design.

15

*Layer 1:* Non-functional poly(styrene-DVB) PHP membranes with controlled porous morphology to exclude large particles such as dust, soil, cells, etc.

*Layer 2:* A layer of PHP modified with pepsin for prevention of protein fouling.

*Layer 3:* A layer containing a sensing species.

20

Ionophores, ligands and enzymes are incorporated either by physical entrapment or chemical modification to provide and ensure an optimum supply of ligand material. The polymer was made conducting by incorporation of graphite particles, metallic particles or electron mediators such as ferrocene or TTF-  
25 TCNQ. PolyHIPE materials are selected according to the porosity of the material, whereby sensors are constructed which can be used in adverse media such as industrial processing media, whole blood and brackish water.

Preparation of modified polyHIPE

Styrene (4.5 g), divinylbenzene (0.5 g), ligand (ca 0.02g) and Span 80 (1.0 g) were mixed in a three-necked round-bottomed flask with a D-shaped PTFE paddle connected to an overhead stirrer at 300 rpm under nitrogen. To this was added dropwise 45ml of an aqueous solution containing potassium persulfate (0.1 g) and calcium chloride dihydrate (0.5 g). The HIPE developed as a viscous white fluid, which was stirred for a further hour following addition of the aqueous phase. It was then transferred to PTFE moulds which were heated at 60 °C in an oven for 48 hours. The resulting modified PolyHIPE membranes were immersed in de-ionised water then *iso*-propyl alcohol for 24 hours each, to remove salts and surfactant respectively, and were dried *in vacuo* to constant mass.

#### Potentiometric Sensors

To prepare potentiometric sensor devices, the lower sensing layer is a conventional nonporous polymer membrane e.g. PVC, containing the ligand and plasticiser. The plasticiser makes the membrane flexible and solvates the ligand. It is necessary to have a non-porous layer in potentiometric mode as equilibrium conditions need to be established, which will only occur if the analyte is transported through the membrane to the inner filling solution or electrode by the ligand.

However, porous PolyHIPE materials are used to prepare the other layers as shown in fig. 2. Ligands used are valinomycin to detect clinically relevant K<sup>+</sup> and a lead ionophore such as N,N'-dioctadecyl-N,N'-dipropyl-3,6-dioxaoctandiamide (Fluka) to detect Pb<sup>2+</sup>, a source of environmental pollution.

Ionophore modified PolyHIPE provides a new material for improving the analytical applications of current ion-selective electrode systems. The optimum

thickness for each layer is determined by iteration. Once prepared, the composite membrane is cut to the appropriate size and fitted in a Philips electrode-type body. The inner-filling solution is the appropriately buffered analyte solution in a sol-gel matrix. This configuration helps in preventing the leaching of the 5 electrolyte from the inner-filling solution thereby achieving the conditions required to obtain pico-molar limits of detection.

Comparisons are made with traditional potentiometric ion-selective electrodes. Membranes containing ionophores for hydrophilic (e.g.  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ , etc.) 10 and hydrophobic (e.g. chiral and achiral aryl and alkyl ammonium) cations are also prepared by corresponding methods.

### Amperometric Sensors

In amperometric mode, detection occurs via a redox reaction of the analyte at the 15 electrode surface; consequently, equilibrium conditions are not necessary. For this reason porous PolyHIPE is used instead of non-porous PVC membrane as the substrate for the sensing layer, which allows rapid access of the analyte to the electrode surface giving rapid detection. Conducting species such as graphite particles are incorporated into the PHP to increase the detection signal or a 20 conducting polymer such as polypyrrole is coated onto the inner surface of the base PHP material, using a procedure described by Ruckenstein and coworkers<sup>v</sup>. Sensors are made using the  $\text{Pb}^{2+}$  ionophore ligand mentioned above; also using modifying agents to detect other environmental pollutant cations such as  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$ .

25

### Example 3 – Application to Industrial Process Control

Sensor of Example 2 above are made up as arrays and installed in the reaction chamber and effluent streams of an industrial process using a heavy metal.

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Periodic stripping voltammetry linked by suitable instrumentation and software gives real time readings of heavy metal concentration in each case, whereby adjustment can be made of the inlet rate of metal for optimum stoichiometric reaction or for minimum effluent content.

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CLAIMS

1. A sensor for electrochemically or optically determining a desired species, the sensor comprising at least one electrode or optical path, wherein the 5 electrode or optical path is modified for specific accumulation of the desired species, by means of a modifying agent which is adapted to selectively and substantially reversibly bind the species to be detected and to release the species at a characteristic voltage or give an absorbance measurement at a characteristic optical wavelength, and wherein the 10 modifying agent is associated with at least part of the surface of the electrode or optical path by physical or chemical anchoring.
2. A sensor in accordance with claim 1 wherein the sensor is electrochemical.
- 15 3. A sensor in accordance with claim 2 wherein the sensor is amperometric.
4. A sensor in accordance with claim 2 wherein the sensor is potentiometric.
- 20 5. A sensor in accordance with any preceding claim further comprising exclusion means for excluding non desired species from the surface of the electrode or optical source, such means being selected from size-specific exclusion means about the electrode or optical source and remote therefrom, to prevent access of large particles such as dust, soil, cells etc, or exclusion agents about the electrode or optical source and remote 25 therefrom.
6. A sensor in accordance with any preceding claim further comprising an additional layer of material with tuneable porosity, mechanical stability and resistance to fouling.

7. A sensor in accordance with claim 6 wherein the additional layer is a porous polymer.
- 5 8. A sensor in accordance with claim 7 wherein the additional layer is polyHIPE.
9. A sensor in accordance with any preceding claim wherein the modifying agent and any exclusion agents present are chemically anchored to the 10 electrode or optical source.
10. A sensor in accordance with claim 9 wherein the chemical anchors are selected from lipophilic groups such as C<sub>4-20</sub> aliphatic or alicyclic saturated or unsaturated hydrocarbon groups for example C<sub>8</sub> octyl groups 15 and the like.
11. A sensor in accordance with any preceding claim wherein the modifying agent and any exclusion agents present are physically anchored to the electrode or optical source.
- 20 12. A sensor in accordance with claim 11 wherein the physical anchors are selected from means comprising a porous open or closed cell membrane or film applied at or about the surface and having an electrode or optical source modifying agent and/or any exclusion agent anchored in turn 25 within its pores.
13. A sensor in accordance with claim 12 wherein modifying agent and/or any exclusion agent is physically anchored within the pores by use of pore interconnects of diameter which preclude release of modifying agent but 30 allow access of the species to be detected.

14. A sensor in accordance with claim 12 or claim 13 wherein modifying agent and/or any exclusion agent is chemically anchored within the pores by use of groups or substituents having an affinity for the porous material.

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15. A sensor in accordance with one of claims 12 to 14 wherein the physical anchoring means comprise porous open cell structures selected from high internal phase emulsion polymers (Poly-HIPE or PHP), solgels, electrically conducting or optically transparent zeolites and the like.

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16. A sensor in accordance with claim 15 wherein the physical anchoring means comprises PHP membrane of thickness 1 micron – 5 mm and having pore size in the range up to 100 micron and interconnect size in the range up to 10 micron.

15

17. A sensor in accordance with any preceding claim wherein the modifying agent is selected from the group comprising ionophores, ligands, enzymes and other multi-dentate species providing reversible binding by affinity such as charge delocalisation, for example hydrogen bonding, van der Waals forces and the like.

20

18. A sensor in accordance with claim 17 wherein the modifying agent is selected from compounds of formula

25 (R)<sub>n</sub> – A

wherein n is 0 – 2 and R is a lipophilic or other group suitable for stably binding with high affinity to the electrode surface or a surface layer such as polyHIPE or PVC surface membrane; when n is 0, the

ligand is physically anchored, for example caged in membrane pores

and wherein A is a C<sub>1-36</sub> aliphatic or alicyclic saturated or unsaturated or aromatic hydrocarbon moiety optionally including one or more heteroatoms selected from N and S and the like, and optionally substituted by functional groups including halo, hydroxy, cyano, amino, carbonyl, and the like and optionally including one or more ether, amino or thioether linkages and having m binding sites for accumulating a desired cationic species, wherein m is 1 – 6, preferably 1 – 4.

19. A sensor in accordance with claim 17 wherein the sensor is adapted for the detection of chromium species and in particular for the detection of Cr(III) speciated with respect to Cr(VI) and the modifying agent is selected from ligands comprising modified 2, 3 pyridine dicarboxylic acid of general formula I

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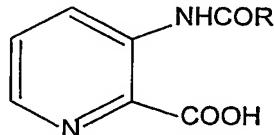


Fig 1

which may optionally be substituted at the 4, 5 and 6 positions by C<sub>1-8</sub> saturated or unsaturated hydrocarbon moieties, halo and the like or fused polycyclic aromatic systems.

20. A sensor in accordance with claim 17 wherein the sensor is adapted for the detection of lead and the modifying agent is selected from ligands

comprising modified N, N' substituted aliphatic diamides such as N,N'-dioctadecyl-N,N'-dipropyl-3,6-dioxaoctandiamide (Fluka).

21. A sensor in accordance with claim 17 wherein the sensor is adapted for the detection of potassium K<sup>+</sup> and the modifying agent is selected from ligands selected from the class of the mycin antibiotics such as valinomycin.
22. A method for the manufacture of a sensor as hereinbefore defined comprising providing at least one electrode or optical path, applying a layer of modifying agent thereabout, and anchoring by chemical or physical means, applying exclusion means thereabout and anchoring, and providing electrical contacts or optical source.
23. The method in accordance with claim 22 applying adsorptive stripping techniques, for species capable of undergoing redox reactions, in that the species to be detected is pre-concentrated on a modifying agent of moderate selectivity by applying a potential opposite to the charge on the analyte, and the potential is reversed to strip the species.
24. The method in accordance with claim 22 or claim 23 wherein modifying agents with appropriate binding constants are first deposited either physically (screen-printed) or chemically attached to the electrode surface.
25. A novel electrode, modified with use of a modifying agent as hereinbefore defined, preferably with use of a Cr (III) selective agent, for Cr (III) detection or with use of a Cr (VI) selective agent, for Cr (VI) detection. Cr (VI) is highly toxic and therefore of environmental concern.

26. A method for species detection with use of a sensor as hereinbefore defined comprising providing or obtaining a sample to be detected in fluid phase, applying an electrical current or optical source and scanning through a pre-determined range to a stripping potential or wavelength, maintaining the current or optical *source* at that potential or *wavelength* and detecting or measuring the current evolved or absorbance, optionally with conversion thereof to indicate concentration of species.

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27. The method in accordance with claim 26 wherein collected samples are first treated by subjecting to ultrasound to break down signal distorting contaminants, and to break down polymerised species to detectable form.

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28. A method for process control of an industrial process for the introduction and reaction of a given species, comprising monitoring the species with use of the sensing method and sensor as hereinbefore defined, in situ in real time operation, periodically obtaining values for the concentration of species in a given location and deriving parameters to maintain or alter the process controls for continued efficient operation.

15

20 29. The method of claim 28 comprising the use of suitable software and instrumentation to receive and process signals and relate to a calibration logarithm to produce concentration values.

25

30. A method for effluent control or contaminant detection with use of the method and sensor as hereinbefore defined.

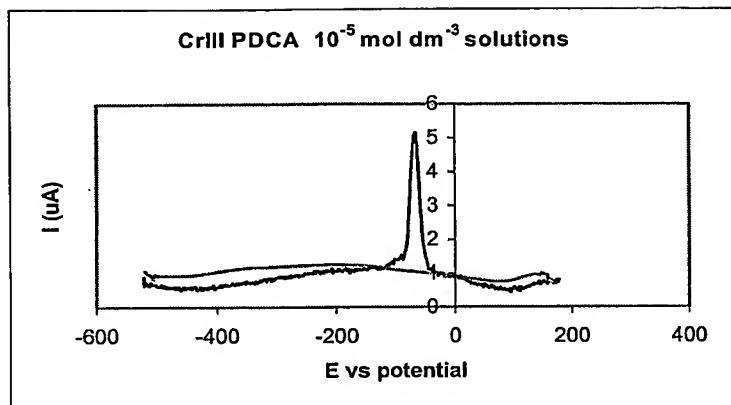
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31. The use of a method or sensor in accordance with any preceding claim for determination and speciation of target species in harsh media such as whole blood and other body fluids, brackish water, industrial environments and the like.

32. The use of a method or sensor in accordance with any preceding claim in electroplating or metal finishing, timber preservation, pigmentation, leather tanning, contaminant detection, nutrient development, heavy metal manufacture and the like.
- 5
33. A sensor or method substantially as hereinbefore described with reference to the accompanying examples and/or drawings.

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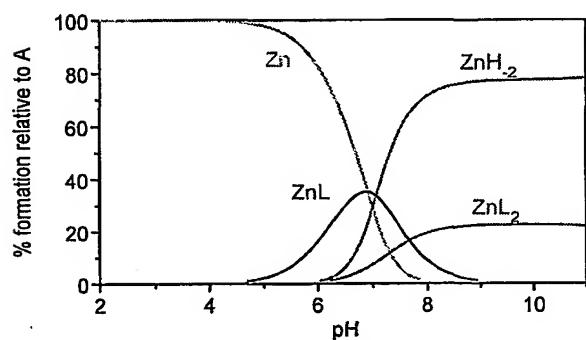
Figure 1



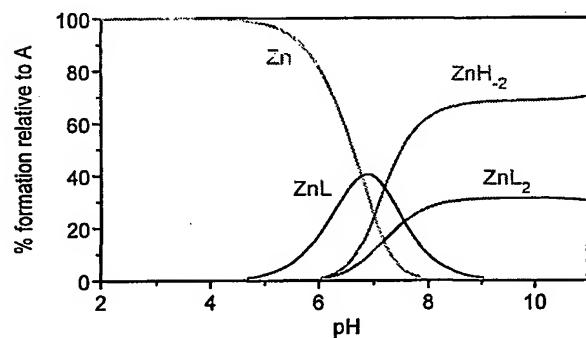
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Figure 2

10mM Zn



5mM Zn



0.1mM Zn

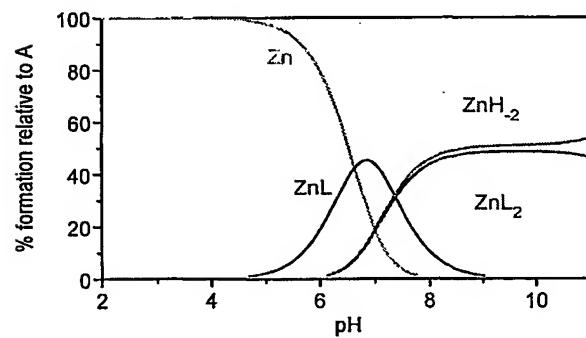


Figure 3

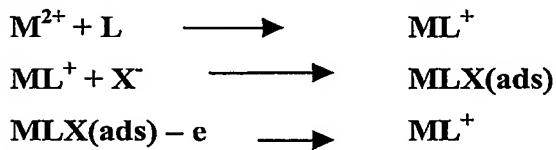
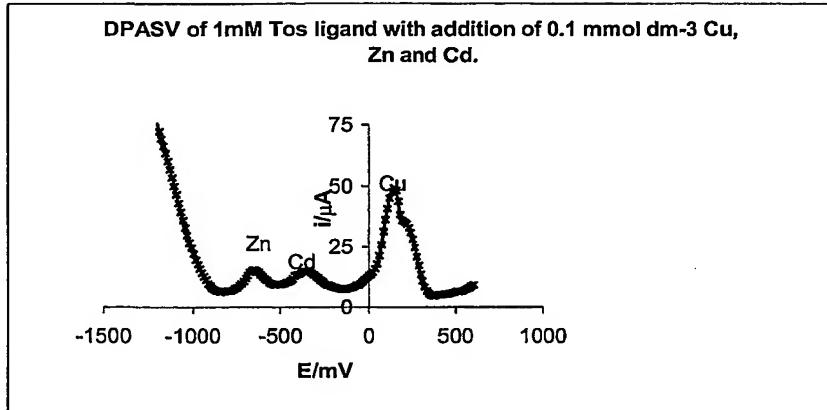


Figure 4

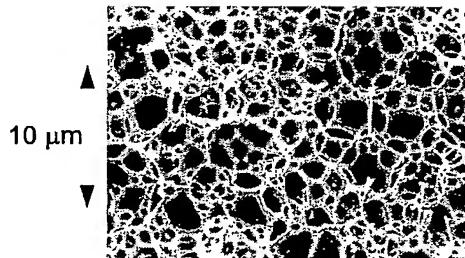
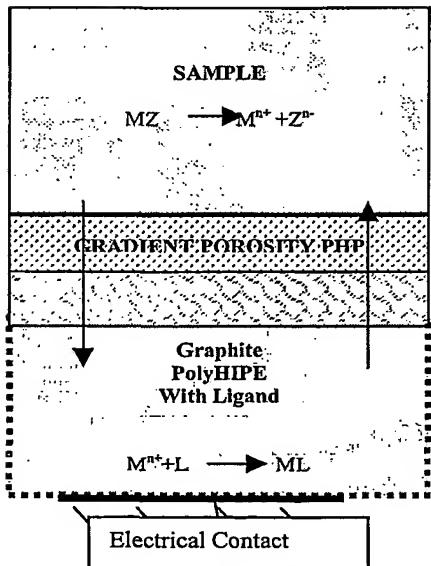


Figure 5



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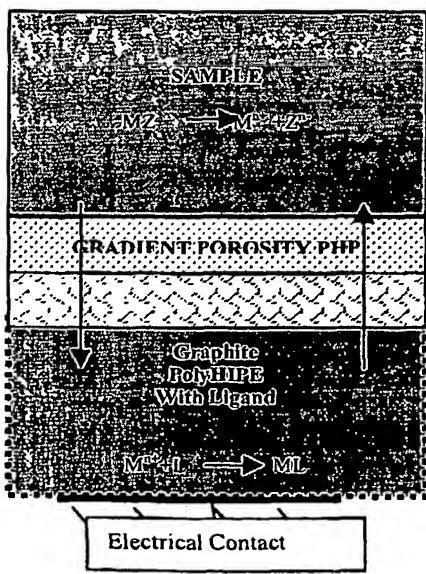
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SESSION AND SENSING METHOD FOR DETECTION AND PROCESS CONTROL



(57) Abstract: A sensor for electrochemically or optically determining a desired species, the sensor comprising at least one electrode or optical path, wherein the electrode or optical path is modified for specific accumulation of the desired species, by means of a modifying agent which is adapted to selectively and substantially reversibly bind the species to be detected and to release the species at a characteristic voltage or give an absorbance measurement at a characteristic optical wavelength, and wherein the modifying agent is associated with at least part of the surface of the electrode or optical path by physical or chemical anchoring. A method of fabricating and using such a sensor, particularly in harsh media such as whole blood and other body fluids, brackish water, industrial environments and the like.

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International Application No  
PCT/GB 02/02035

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 GO1N27/30

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 24840 A (ECOSSENSORS LTD ;MCALLEER JEROME FRANCIS (GB); ACKLAND MARTIN ROBER) 15 August 1996 (1996-08-15) cited in the application page 8, line 9 - line 29; figure 2 ---	1-33
X	US 6 090 269 A (MANDLER DANIEL ET AL) 18 July 2000 (2000-07-18) cited in the application column 1, line 27 -column 2, line 15 ---	1-33
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